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(21) International Application Number: PCT/AU87/00038 (22) International Filing Date: 12 February 1987 (12.02.87) (71) Applicant: THE UNIVERSITY OF MELBOURNE [AU/AU]; Grattan Street, Parkville, VIC 3052 (AU). (72) Inventors: BATEMAN, John, Francis ; 14 Kalimna Street, Balwyn, VIC 3103 (AU). RAMSHAW, John, Alan, Maurice ; 121 Blyth Street, Brunswick, VIC 3056 (AU). PETERS, David, Edward ; Paynters Road, Wonga Park, VIC 3155 (AU). TULLOCH, Pe- ter, Archibald ; 71 Park Drive, Parkville, VIC 3052 (AU). (74) Agent: SANDERCOCK, SMITH & BEADLE; 207 Riversdale Road, Hawthorn, VIC 3122 (AU).		(81) Designated States: BR, KR. Published <i>With international search report.</i>
(54) Title: PRECIPITATION OF COLLAGEN IN TACTOID FORM (57) Abstract Collagen in tactoid form obtained by forming an aqueous solution containing dissolved collagen and a water solu- ble or miscible polymer adapted to precipitate collagen out of solution in the form of tactoids.		

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PRECIPITATION OF COLLAGEN IN
TACTOID FORM

1
2 This invention relates to collagen products.
3 In a particular aspect this invention relates
4 to collagen products made from soluble collagen. A new
5 method by which soluble collagen can be formed into
6 quasi-crystalline structures by precipitation using
7 soluble polymers is described. The use of an aggregate
8 of this quasi-crystalline collagen to form a variety of
9 collagen materials which have improved properties
10 compared with existing collagenous materials is
11 described. Such improved collagen materials have
12 application in various fields including the manufacture,
13 for example, of products for medical use.
14 Collagen is an extremely common protein in the
15 animal kingdom and therefore many uses for products
16 based upon collagen have developed. Many products use
17 collagen in either its native form (i.e. the triple
18 helical structure pre-existing in an animal or human
19 body), or regenerated into this form, or after denaturation
20 of the collagen, in the form of gelatine. Native collagen
21 is used for various products such as in the production
22 of leather from animal skins, or such as the production
23 of sausage casings in which the collagen is finely
24 divided and reformed into the desired structure.
25 There are also many uses of collagen and for items
26 made from collagen in medical fields such as in
27 artificial arteries, veins, tendons, corneas, heart
28 valves, skin, or patches or the like which are used as
29 replacement parts for disease or injury affected parts in
30 humans, or in cosmetic applications such as mammary
31 prostheses or injectable collagen, or in collagen
32 sponges, sutures or haemostat materials which may be
33 used during surgery or in the treatment of disease
34 (Chvapil, 1979). Many of these medical products made from
35 collagen are at present unsatisfactory because of an
36 inability to reproduce the native structure, composition
37 or strength which exists in the normal
38 collagenous tissue or because of the immune response

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1 elicited by the presence of immunogenic collagen or
2 components or other material foreign to the body.

3 In its native form in the body, collagen exists in
4 many types and in the most common of these types, collagen
5 exists as fibrils in which individual collagen
6 molecules are arranged in a staggered overlap
7 structure (Bornstein and Traub, 1979). These fibrils
8 are stabilised and made insoluble by
9 intermolecular crosslinks between the non-helical
10 portions (telopeptides) of adjacent collagen molecules
11 (Bornstein and Traub, 1979). If the collagen from normal,
12 mature tissue is to be made soluble the crosslinks must
13 be broken, for example by digestion with an enzyme such as
14 pepsin.

15 Soluble collagen can be reconstituted in a variety
16 of ordered aggregate forms. Some are fibrous in form,
17 and fibrils in which the collagen is arranged in its
18 native staggered way can be reformed. The rate of the
19 fibril reforming process is enhanced if collagen with
20 intact telopeptides is used. However, results from the
21 use of injectable soluble collagen have shown that the
22 telopeptides lead to an antigenic response in humans;
23 collagen lacking telopeptides is relatively non antigenic
24 (Linsenmayer, 1982) but can still be made to form fibrils.
25 Materials formed by fibril regeneration are often too
26 hydrated and additional methods such as freeze-drying or
27 cell-induced contraction must be used to give a functional
28 product.

29 Other non-native fibrous aggregates, termed
30 FLS collagen, can be formed in which the collagen molecules
31 are arranged in various staggered arrangements with
32 the orientation of the molecules in both directions.

33 Quasi-crystalline aggregates can also be formed.
34 These include very small crystallites of collagen,
35 termed SLS collagen, in which the collagen molecules all
36 have the same orientation, but there is no stagger
37 between molecules. These have been of partial use in
38 deducing the native structure of collagen but SLS collagen

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1 has been of little use in the manufacture of larger
2 structures like biomedical products. Also, quasi-
3 crystalline tactoids of collagen can be prepared, using
4 conditions similar to those used for reconstituting
5 fibrils by heat gelation (Leibovich and Weiss, 1970; Lee
6 and Piez, 1983) but the technique of production is more
7 difficult than the technique described here as it
8 does not involve simple precipitation. In these
9 structures the collagen is arranged in a staggered form
10 similar to native fibrils. In the present work the
11 tactoids are produced by a new procedure,
12 precipitation by soluble, neutral polymers. When collagen
13 is precipitated by other procedures, for example salts,
14 alcohols or heat, amorphous precipitates are formed.

15 DESCRIPTION OF THE INVENTION

16 During a search for more efficient methods of
17 isolating soluble collagen it was found that the addition of
18 water soluble polymers to a solution of collagen resulted
19 in an efficient precipitation of the collagen from
20 solution and the precipitated collagen was found to be much
21 easier to separate from the liquid phase than with
22 precipitates of collagen formed by the use of salts,
23 alcohol or heat. The polymers had other advantages when
24 compared with these previously used precipitants
25 including that they were non-denaturing and did not
26 require removal prior to chromatography or
27 electrophoresis.

28 It was an unexpected finding that the collagen
29 had precipitated in the form of small, needle-
30 like, quasi-crystalline tactoids which were visible under
31 the light microscope.

32 It was a further unexpected discovery that the
33 tactoids could be induced to form into larger assemblages
34 either by allowing the suspension to mature for a period
35 of time or by mechanical action, and that the tactoids or
36 their assemblages could be formed into shapes.

37 Accordingly, the present invention provides a method
38 of producing a collagen product comprising forming an

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1 aqueous solution containing dissolved collagen and a water
2 soluble or miscible polymer adapted to precipitate the
3 collagen out of solution in the form of tactoids.

4 The pH of the said solution is preferably 3.5-10
5 more preferably 5-8 with 7-8 being still more preferred and
6 about 7.5 being most preferred.

7 The collagen precipitate may be left in the form of
8 a paste or slurry and used in this form or after
9 concentration by any one of the methods gravitational
10 precipitation, filtration, centrifugation or the like. The
11 precipitate may be crosslinked, tanned or stabilised by
12 one or more of chemical, physical or biochemical methods
13 either before or after it has been concentrated.
14 Crosslinking, tanning or stabilisation applied to the
15 precipitate before concentration makes the tactoids
16 resistant to deforming actions such as heating,
17 pressure or biochemical degradation. Crosslinking,
18 tanning or stabilisation applied to the precipitate
19 after concentration causes the structure formed
20 during the concentration process to become more stable.

21 The so precipitated collagen may also be formed,
22 for example, into a synthetic body part. Such forming
23 into a synthetic body part may be effected by
24 gravitational precipitation, filtration, centrifugation,
25 moulding, pressing, shaping or any other way or combination
26 of ways.

27 Shapes which may be prepared include sheets,
28 tubes, strings and rods.

29 It has been found particularly desirable to form the
30 so precipitated collagen into sheets for use as
31 synthetic dressings for wounds and into tubes for use as
32 synthetic tubular body parts. The sheets can be
33 formed by centrifugation in a large basket centrifuge or
34 the like or by gravitational precipitation or filtration.
35 Other methods of producing the sheets are also possible.
36 A more compacted sheet is produced by centrifugation
37 in comparison with gravitational precipitation or
38 filtration. Tubes can also be prepared by centrifugation

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1 or by casting, moulding or shaping.

2 The collagen may be precipitated onto a
3 suitable substrate to form a composite material. Such a
4 substrate, onto which the collagen is precipitated, may have
5 the form of a particular body part or biomedical product.

6 The substrate may take the form of a matrix.

7 The substrate may take the form of a plastic or
8 other synthetic surface in the form of a sheet, tube or
9 mesh, onto which the collagen is directly deposited
10 forming a collagenous coating.

11 The substrate may also take the form of a composite,
12 for example, various synthetic layers bonded to an
13 artificially or naturally-produced matrix.

14 These collagen coated substrates may also be
15 chemically modified. For example, glutaraldehyde or
16 similar chemicals may be used to stabilise the matrix.

17 The collagen of the present invention may be used as a
18 paste or slurry. Such a paste or slurry would have a number
19 of applications including as an implant material such as in
20 the form of an injectable medium for use in cosmetic
21 surgery. Such a slurry may be stabilized chemically such as
22 by glutaraldehyde or irradiation. Such as with gamma
23 radiation. The concentration of this tactoidal collagen in
24 the paste or slurry is preferably not less than 10 mgm/ml,
25 more preferably not less than 30 mgm/ml and most preferably
26 not less than 40 mgm/ml.

27 The collagen useful for forming the collagen products
28 of this invention includes collagen derived from hides,
29 skins or other collagen containing organs or tissues of
30 humans or other vertebrates or invertebrates and includes
31 collagens of one type or mixtures of types. Soluble
32 collagen can be prepared by enzymic treatment of collagen
33 from those sources. Suitable enzymes include pepsin.

34 The collagen may also be derived from the culture
35 medium of cells, tissues or organs grown in cell- or tissue-
36 culture. The culture medium used to produce the collagen
37 may be a culture medium from cell or tissue culture
38 derived from a person for whom a synthetic body part is

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1 to be produced; it is believed that doing this will
2 substantially reduce the likelihood of rejection.
3 Further, it is also possible that a substrate may be
4 introduced into the culture medium such that collagen and
5 other components will be directly produced thereon.
6 Such a substrate may have the form of a particular body
7 part or biomedical product desired. The substrate may
8 take the form of a matrix. The substrate may take the form
9 of a plastic or other synthetic surface in the form of a
10 sheet, tube or mesh, onto which the collagen and other
11 components are directly deposited forming a collagenous
12 coating. The substrate may be formed from aggregates of
13 tactoidal collagen of this invention.

14 The water soluble or miscible polymer is preferably
15 a neutral polymer. Such polymers may be at least one of
16 the synthetic polymers polyvinyl alcohol, polyethylene
17 oxide, polyvinylpyrrolidinone, polyacrylamide, polyethylene
18 glycol, polypropylene glycol, polyvinyl methyl ether,
19 maleic anhydride copolymers and the like; or at least one
20 of the modified, natural, neutral polymers hydroxyethyl
21 starches, methyl cellulose, hydroxymethyl cellulose,
22 hydroxyethyl cellulose, hydroxypropyl cellulose or the like;
23 or at least one of the natural neutral polymers
24 agarose, dextrans, dextrans, starches, pectins,
25 alginates and the like. Mixtures of such polymers
26 may be used and the molecular weight of the polymer
27 or polymers can vary over a wide range provided the
28 polymer remains soluble or miscible with water.

29 This list of polymers is not exhaustive as the
30 important factor is the use of a water soluble polymer or
31 polymers to precipitate the collagen. Neutral water
32 soluble or miscible polymers are preferable but charged,
33 water soluble polymers may also be used particularly
34 if they are only mildly charged.

35 The precipitate of collagen is generally found to
36 be improved if it is allowed to stand in said solution.
37 Such standing is preferable for a period of one hour to six
38 months with one day to one month being more preferred.

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1 Such standing is effected at temperatures between
2 the denaturation temperature of the collagen and the
3 freezing point of the solution; preferably at between zero
4 and 20°C; more preferably between zero and 10°C.

5 If desired, added materials such as
6 plasticisers, colourants, biologically active
7 materials such as proteoglycans or
8 glycosaminoglycans, proteins, other extracellular
9 products, hormones, growth factors, antibiotics and agents
10 which affect wound healing or have other beneficial
11 effects, ionic strength modifiers such as salts, or
12 solids such as insoluble collagen or the like may be
13 included with the so precipitated collagen and
14 incorporated into material made from the collagen. These
15 added materials may also be incorporated into the
16 solution of soluble collagen before addition of the
17 polymer or otherwise incorporated into material made
18 from the collagen. Charged, water soluble or water
19 miscible polymers may be used as part of a mixture with
20 the neutral polymer or polymers and added to the soluble
21 collagen with the neutral polymer solution. These
22 charged polymers may be used to modify the properties of
23 the soluble collagen solution or the material made from
24 the precipitated collagen.

25 The collagen product of this invention may be
26 chemically or biochemically stabilised. Biochemical
27 stabilisation may be effected by enzymes such as
28 lysyl oxidase. Chemical stabilisation may be effected
29 by tanning agents, syntans, other cross-linking agents
30 or chemical modifiers of collagen. Of particular
31 interest are stabilisers which limit proteolysis
32 or the immunogenicity of the collagen.
33 Glutaraldehyde is a stabiliser of particular interest.
34 The product may also be stabilised by dehydration by mild
35 heat, water miscible solvents, critical point drying or the
36 like. Such stabilisation may be performed before or after a
37 shaping operation. The collagen product of this
38 invention may be sterilised chemically or by irradiation.

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1 Chemical sterilisation may be conducted by means of
2 suitable solutions of sterilising materials such as
3 glutaraldehyde from between 0.5% to 5% concentration.
4 The product may be stored in solutions of sterilant
5 until required for use. Sterilisation by means of
6 irradiation can be conducted by exposing the collagen
7 product of this invention to gamma rays from a suitable
8 source. From 0.5 to 5 Mrads of irradiation may be used,
9 preferably 2.5 Mrads of gamma ray irradiation is suitable
10 for satisfactory sterilisation of the product.

11 The tactoids formed by precipitation of the
12 soluble collagen in this invention are useful in
13 production of synthetic body parts, and other materials
14 for medical or veterinary applications. The collagen
15 tactoids or tactoid assemblages could be stabilised by
16 chemical or biochemical techniques or could be formed
17 into various useful shapes and then stabilised. The
18 tactoidal collagen has potential application in many
19 areas such as the manufacture of collagen sponges or
20 haemostatic agents, of dressings, of membranes, of skin,
21 of tubes and the like and in the treatment of
22 disease such as periodontal disease. The tactoidal
23 collagen can also be used in conjunction with other
24 structural type materials to form composite materials
25 with different properties. For example, a tube of
26 tactoidal collagen can be covered with a woven or knitted
27 mesh of fibre such as Dacron to give the tube additional
28 strength. Alternatively, the tactoidal collagen can be
29 formed into a tube surrounding the mesh to give a more
30 intimate contact with the mesh and better properties. To
31 better utilise the properties of the tactoidal
32 collagen in the formation of artificial body parts it is
33 possible to arrange the tactoids in a preferred
34 orientation by the application of an electric field or
35 by means of mechanical action. Materials made from the
36 oriented tactoids may have beneficial effects in the
37 healing of wounds. Many other methods of utilising
38 the tactoidal collagen in a variety of shapes and forms

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1 and in conjunction with diverse other materials can be
2 envisaged.

3 The product of this invention also has application
4 in areas outside medical and veterinary products
5 including plastics, fabric, leather or as composites or the
6 like.

7 The present invention also includes such
8 collagen products and articles produced therefrom.

9 The collagen products of this invention have
10 advantages over presently available products. These
11 include, low immunogenicity, ease of preparation, high
12 collagen content, and strength.

13 The following examples illustrate the invention.

14 EXAMPLE 1

15 Type I collagen was solubilised and extracted from
16 foetal calfskin by pepsin digestion and purified by
17 fractional salt precipitation according to the method
18 of Trelstad et al.(1967). This purified collagen was
19 dissolved in 200 mM Tris-HCl buffer pH 7.5 at 4°C and at
20 a concentration of 10 mg/ml. Polyethylene glycol (PEG)
21 4000 was then added to produce a final concentration of
22 2.5% (w/v). A precipitate of tactoidal collagen formed
23 which settled to the bottom of the container after
24 standing at 4°C for a few hours or could be concentrated
25 by filtration or centrifugation.

26 EXAMPLE 2

27 As for Example 1 except that the concentration
28 of the collagen was 1 mg/ml.

29 EXAMPLE 3

30 As for Example 2 except that PEG 400 to a final
31 concentration of 3.5% (w/v) was used to precipitate the
32 collagen.

33 EXAMPLE 4

34 Type III collagen, solubilised and extracted as in
35 Example 1, was dissolved at a concentration of 1 mg/ml in
36 200mM Tris- HCl buffer pH7.6 at 4°C. PEG 400 was added to
37 the solution to a final concentration of 4.0% (w/v) and
38 the precipitate of tactoidal collagen formed.

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1 EXAMPLE 5

2 As for Example 4 except that a final concentration of
3 2.5% (w/v) PEG 4000 was used.

4 EXAMPLE 6

5 Type II collagen was isolated by the method of
6 Trelstad et al. (1976) from bovine articular
7 cartilage by pepsin solubilisation and fractional
8 salt precipitation. The purified type II collagen was
9 dissolved in 200 mM Tris- HCl buffer at pH 7.6 at 4°C and
10 at a concentration of 1 mg/ml. PEG 400 was then added to
11 produce a final concentration of 3.0% (w/v). The
12 precipitate of tactoidal collagen formed as in Examples
13 above.

14 EXAMPLE 7

15 As for Example 6 except that PEG 4000 was added to a
16 final concentration of 2.0% (w/v).

17 EXAMPLE 8

18 As for Example 1 except that PEG 1000 to a
19 final concentration of 5% (w/v) was used to
20 precipitate the collagen.

21 EXAMPLE 9

22 As for Example 1 except that PEG 10000 to a
23 final concentration of 5% (w/v) was used to
24 precipitate the collagen.

25 EXAMPLE 10

26 The suspension of tactoidal collagen from Example
27 1 was stored at 4°C for 4 weeks and collected on
28 Whatman No. 1 filter paper in a 125 mm diameter basket
29 centrifuge rotating at 4000 rpm. The resulting collagen
30 sheet was removed from the centrifuge and separated from
31 the filter paper. The collagen sheet was found to have
32 properties similar to those of a thick, wet paper tissue
33 and to be suitable for assisting in the healing of open
34 skin wounds.

35 EXAMPLE 11

36 The collagen sheet, prepared as in Example 10, was
37 tanned using a solution of 0.01% glutaraldehyde for 18
38 hours. After drying the sheet was found to have a

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1 tensile strength of 6.2N/sq cm and an elongation of 12%
2 at a moisture content of 16%.

3 EXAMPLE 12

4 The collagen sheet, prepared as in Example 10 was
5 sealed in a polyethylene bag and subjected to 2.5Mrads
6 of gamma ray irradiation. The sheet was found to have
7 been sterilised and to have improved tensile properties
8 over those of the sheet in Example 10.

9 EXAMPLE 13

10 As for Example 2 except that the buffer was at pH5.

11 EXAMPLE 14

12 As for Example 1 except that the collagen extracted
13 from foetal calfskin was not purified by fraction
14 salt precipitation but was used as a crude extract and that
15 5% PEG 4000 was used.

16 EXAMPLE 15

17 As for Example 14 except that 5% polyvinyl alcohol was
18 used.

19 EXAMPLE 16

20 As for Example 14 except that 5% dextran of 10,000
21 average molecular weight was used.

22 EXAMPLE 17

23 As for Example 14 except that 5% dextran of 40,000
24 average molecular weight was used.

25 EXAMPLE 18

26 A collagen sheet prepared as in Example 10 was rolled
27 into a tube and then stabilized by tanning using a solution
28 of 0.01% glutaraldehyde for 18 hours.

29 EXAMPLE 19

30 A collagen sheet prepared as in Example 10 was dried by
31 critical point drying using liquid carbon dioxide.

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13 Separation of native types I, II and III by differential
14 precipitation.
- 15 Modifications and adaptations may be made to the
16 above described without departing from the spirit and scope
17 of this invention which includes every novel feature and
18 combination of features disclosed herein.

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1 CLAIMS:

2 1. Collagen in tactoid form obtained by forming an aqueous
3 solution containing dissolved collagen and a water soluble
4 or miscible polymer adapted to precipitate collagen out of
5 solution in the form of tactoids.

6 2. A method of producing a collagen product comprising
7 forming an aqueous solution containing dissolved collagen
8 and a water soluble or miscible polymer adapted to
9 precipitate the collagen out of solution in the form of
10 tactoids.

11 3. A method of producing a collagen product as claimed in
12 claim 2, wherein the pH of said solution is 3.5 - 10.

13 4. A method of producing a collagen product as claimed in
14 claim 2, wherein the pH of said solution is 7 - 8.

15 5. A method of producing a collagen product as claimed in
16 any one of claims 2 - 4, including forming the thus formed
17 precipitate to a shape.

18 6. A method of producing a collagen product as claimed in
19 any one of claims 2 - 5, including precipitating the
20 collagen onto a pre-shaped substrate.

21 7. A method of producing a collagen product as claimed in
22 claim 6, wherein the substrate has the form of a body part.

23 8. A method of producing a collagen product as claimed in
24 claim 6, wherein the substrate is itself formed of collagen
25 in the form of tactoids.

26 9. A method of producing a collagen product as claimed in
27 claim 5, wherein prior to forming said precipitate to a
28 shape the precipitate is permitted to stand in said solution
29 for a period of greater than 1 hour.

30 10. A method of producing a collagen product as claimed in
31 claim 9, wherein the temperature of standing is from 0 -
32 20°C.

33 11. A method of producing a collagen product as claimed in
34 any one of claims 2 - 10, and including the step of
35 chemically or biochemically stabilizing the collagen so
36 formed.

37 12. A method of producing a collagen product as claimed in
38 any one of claims 2 - 11, wherein the dissolved collagen is

1 derived from cell or tissue culturing.

2 13. A method of producing a collagen product as claimed in
3 any one of claims 2 - 12, wherein said water soluble or
4 miscible polymer is selected from polyvinyl alcohol,
5 polyethylene oxide, polyvinylpyrrolidinone, polyacrylamide,
6 polyethylene glycol, polypropylene glycol, polyvinyl methyl
7 ether, maleic anhydride copolymers and the like.

8 14. A method of producing a collagen product as claimed in
9 any one of claims 2 - 12, wherein said water soluble or
10 miscible polymer is selected from hydroxyethyl starches,
11 methyl cellulose, hydroxymethyl cellulose, hydroxyethyl
12 cellulose, hydroxypropyl cellulose or the like.

13 15. A method of producing a collagen product as claimed in
14 any one of claims 2 - 12, wherein said water soluble or
15 miscible polymer is selected from agarose, dextrans,
16 dextrans, starches, pectins, alginates and the like.

17 16. Collagen as claimed in claim 1 and in admixture with a
18 biologically active material.

19 17. Collagen as claimed in claim 1 and in the form of a
20 synthetic body part.

21 18. Collagen as claimed in claim 1 and precipitated onto a
22 shaped substrate.

23 19. Collagen as claimed in claim 17 and in the form of a
24 sheet or tube.

25 20. Collagen as claimed in claim 1 and in the form of a
26 slurry or paste.

27 21. Collagen as claimed in claim 20 and containing at least
28 10 mgm/ml of collagen.

29 22. A method of producing a collagen product substantially
30 as hereinbefore described with reference to any one of the
31 Examples.

32 23. Collagen in tactoid form substantially as hereinbefore
33 described with reference to any one of the Examples.

34 24. The articles, things, parts, elements, steps, features,
35 methods, processes, compounds and compositions referred to
36 or indicated in the specification and/or claims of the
37 application individually or collectively, and any and all
38 combinations of any two or more of such.

INTERNATIONAL SEARCH REPORT

International Application No PCT/AU 87/00038

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁸

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl. ⁴ A61L 27/00; C07K 15/12, 15/20; C08J 3/14; C08L 89/00, 89/06

II. FIELDS SEARCHED

Minimum Documentation Searched ⁷

Classification System

Classification Symbols

IPC

A61L 27/00; C07K 15/12, 15/20; C08J 3/14; C08L 89/00, 89/06

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁸

AU : IPC as above, Australian Classification 47.72

III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹

Category ¹⁰ Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹² Relevant to Claim No. ¹³

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IV. CERTIFICATION

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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 87/00038

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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END OF ANNEX